sensory neurons can be targets of LPS, raising the possibility that they may also contribute to trigger and regulate innate immune responses.

1428-Pos Board B379

The Brite Side of TRPV1: Novel Role in Browning of White Adipocytes

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Obesity foreshadows metabolic syndrome. Sedentary life style and high calorie intake lead to obesity. Therefore, a strategy to prevent obesity and facilitate weight-loss is an urgent need. In an effort to achieve this goal, we report that dietary capsaicin (CAP; 0.01%) inhibited weight gain in high fat diet (HFD; 60% calories from fat)-fed wild type mice but not those genetically lacking transient receptor potential vanilloid 1 ($TRPV1^{-/-}$), without modifying *ad libitum* food or water intake. HFD inhibited TRPV1 expression, activity and facilitated fat accumulation in white adipose tissue (WAT) while dietary CAP antagonized the effects of HFD. Furthermore, introduction of CAP in diet suppressed HFD-induced weight gain in wild type mice. Analyses of mechanisms by which CAP antagonized HFD-induced obesity revealed that HFD suppressed TRPV1 expression and activity in adipocytes and CAP ablated this. Also, CAP significantly increased the expression of (1) brown fat marker genes - uncoupling protein-1, bone morphogenetic protein 8b and peroxysome proliferator activated receptor gamma (PPAR) coactivator-1 (PGC-1); (2) sirtuin 1 (SiRT1; NAD-dependent protein deacetylase - a gene that increases fat metabolism) and (3) PRDM-16 (a gene that regulates browning of white fat and promotes energy expenditure) in WAT of wild type but not $TRPV1^{-/-}$ mice. Consistently, dietary CAP increased metabolic activity of wild type mice. The increase in SiRT1 was associated with a concurrent decrease in acetylated PPAR in inguinal and epididymal adipose tissues, which is important for the recruitment of PRDM-16 to PPAR to induce browning of white adipose tissues. CAP also increased the expression of SiRT1 and PRDM-16 in brown adipose tissue. Collectively, we demonstrate that dietary CAP antagonizes obesity by stimulating the browning of WAT. Our work uncovers the emergence of TRPV1 agonists as new drug candidates to combat obesity.

Ligand-gated Channels I

1429-Pos Board B380

Protons Potentiate GluN1/GluN3A Glycinergic NMDA Receptor Currents

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GluN1 and GluN3 subunits of the N-methyl-D-aspartate receptor family form tetrameric cation-permeable channels that are gated by glycine alone and are insensitive to glutamate. They are expressed primarily during early development and their role in cellular physiology is unknown. One hypothesis states that their tonic activation by basal levels of brain glycine controls cellular excitability. Notably, GluN1/GluN3A receptors are selectively up-regulated following ischemia, while 'classical' GluN2A- and GluN2B-containing receptors are down-regulated and also strongly inhibited by ischemic acidification. We found that extracellular protons strongly potentiated peak glycine-elicited currents from recombinant GluN1/GluN3A receptors (Ipk, 10-fold at pH 6.8 versus pH 8.0) with a half maximal effect in the physiologic range (EC50 = pH 7.1 ± 0.03). The time-course of current desensitization was also significantly prolonged (2-fold at pH 6.8 versus pH 8.0) and the recovery from desensitization was accelerated (2-fold at pH 6.8 versus pH 7.4). In addition, extracellular protons decreased receptor sensitivity to the agonist glycine (EC50, 48 ± 6 μM at pH 7.4 versus 83 ± 3 μM at pH 6.8) and to the endogenous potentiator Zn2+ (EC50, 32 ± 3 μM at pH 7.4 versus 185 ± 26 μM at pH 6.8). Importantly, we found that extracellular acidification during glycine-elicited steady-state activity produced a large transient influx of positive charge (Ipk, 8-fold at pH 6.8 versus pH 7.4) and increased steady-state activity (Iss/Ipk, 0.03 ± 0.01 at pH 7.4 versus 0.5 ± 0.2 at pH 6.8) which depolarized the membrane substantially (−23 ± 5 mV at pH 7.4 versus −13 ± 3 mV at pH 6.8). Taken together, these results indicate that small pH fluctuations potently modulate GluN1/GluN3A receptor currents and that protons may play a novel positive modulatory role at GluN1/GluN3A receptors in vivo by increasing cellular excitability.

1430-Pos Board B381

Investigations of the Structural Mechanism of Modulation of the NMDA Receptor

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The NMDA receptor, one of the three main types of glutamate receptors, is involved in learning and memory formation. Upon activation by the agonists, the NMDA receptor forms a cation selective pore. The receptor is an obligate heterotetramer typically composed of GluN1 and GluN2 subunits. The GluN2 subunits can be one of four subtypes (A-D). Each subunit is organized into distinct domains, the intracellular carboxy-terminal domain, the transmembrane pore forming region, and extracellularly the agonist binding domain and the amino-terminal domain (ATD). The ATDs of the NMDA receptor contain the binding site for a number of modulators. Inhibitors and potentiators bind the ATDs with specificity for a particular GluN2 subtype. Zinc inhibits the receptor and has highest affinity for the GluN2A subtype and intermediate affinity for the GluN2B subtype. The synthetic compound ifenprodil inhibits receptors that contain the GluN2B subtype, and spermine potentiates receptors that also contain the GluN2B subtype. Extensive studies have focused on the mechanism of zinc inhibition, and previous work from our lab and others has shown that zinc inhibition proceeds via a cleft-closure conformational change. To determine if the mechanism employed by zinc was a common mechanism of inhibition, we used luminescence resonance energy transfer to map the conformational changes that the receptor undergoes upon binding of ifenprodil in GluN2B inhibition. Additionally, we monitored the conformational changes when the potentiator spermine binds. Interestingly, spermine potentiation of agonist-evoked current in GluN1-GluN2B containing receptors seems to proceed through an opposite structural mechanism to inhibition; both the GluN1 and GluN2B ATDs seem to be stabilized in an open conformational state. Additionally, the data suggest that the lower lobe of the GluN2 ATD twists in addition to moving towards or away from the upper lobe of the ATD.

1431-Pos Board B382

Effects of External and Internal Ca^{2+} on Unitary NMDA Receptor Properties

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N-Methyl-D-aspartate (NMDA) receptors are glutamate- and glycine-gated channels widely expressed throughout the central nervous system. When active, they generate electric and biochemical signals by fluxing Na^+ and Ca^{2+} into the post-synaptic cell. The NMDA receptor-mediated Ca^{2+} transient initiates internal cascades that result in synaptic plasticity and excitotoxicity. In turn, Ca^{2+} regulates channel activity directly and indirectly through allosteric and 2nd messenger mechanisms that are poorly understood. We investigated the effects of external and internal Ca^{2+} concentrations on recombinant GluN1/GluN2A (N1/2A) receptors using single-channel current recordings, statistical analysis, and kinetic modeling. Increasing concentrations of extracellular Ca^{2+} reduced the unitary channel conductance from 75.9 ± 1.4 pS in 0 mM $[Ca^{2+}]_e$, to 55.2 ± 1.6 pS in 1.8 mM $[Ca^{2+}]_e$, and to 11.8 ± 0.7 pS in 75 mM $[Ca^{2+}]_e$. Importantly, receptors lacking the intracellular C-terminal domain (CTD) of the N1 subunit ($N1^{\Delta}$/2A) but not the 2A subunit (N1/$2A^{\Delta}$) exhibited higher unitary conductance in the absence of external Ca^{2+} ($N1^{\Delta}$/2A γ = 83.3 ± 1.0 pS, N1/$2A^{\Delta}$ γ = 79.2 ± 1.4 pS). This is consistent with a role for the N1 CTD in setting channel conductance for Na^+. However, in the presence of physiological external Ca^{2+} (1.8 mM), both exhibited $N1^{\Delta}$/2A (γ = 50.4 ± 1.2 pS, $p<0.05$) and N1/$2A^{\Delta}$ (γ = 60.8 ± 2.0 pS, $p<0.05$) channels had a lower conductance relative to wild-type. Based on these novel results, we asked whether this Ca^{2+}-dependent regulation of conductance depends on calmodulin binding to either the C0 or C1 cassettes of N1. Furthermore, we asked whether local Ca^{2+} influx through the NMDA receptor pore is sufficient for this regulation. Our results help to further unravel the Ca^{2+}-dependent processes that control the properties of individual NMDA receptor channels.

1432-Pos Board B383

NMDA Receptor smFRET Studies Reveal Role of Dynamics of the Agonist-Binding Domain in Mediating Agonist Efficacy

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Ionotropic glutamate receptors are tetrameric ligand-gated ion channels which mediate the majority of excitatory neurotransmission in the central nervous system. This family is subdivided into three classes: the AMPA receptor, the kainate receptor, and the NMDA receptor. Extent of cleft closure of the agonist-binding domain is one mechanism by which the agonist mediates channel activity for a number of the glutamate receptor subtypes. However, only an open-cleft or a closed-cleft conformation has seen in crystal structures of the glycine-binding GluN1 subunit of the NMDA receptor, and no partially-closed cleft states have been observed. Here, we have used single molecule